THE INHERITANCE OF MATING TYPE AT ENDOMIXIS AND DURING VEGETATIVE REPRODUCTION IN PARAMECIUM AURELIA

A dissertation submitted to the Board of University Studies of the Johns Hopkins University in conformity with the requirement for the degree of Doctor of Philosophy

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1938

Baltimore

Reprinted from
Proc. Nat. Acad. Sci. 23:469-474, 1937
Genetics 24:49-58, 1939
J. Exp. Zool. 81:165-179, 1939

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Reprinted from the Proceedings of the National Academy of Sciences,
Vol. 23, No. 9, pp. 469–474. Sept., 1937.

THE INHERITANCE OF SEX AT ENDOMIXIS IN PARAMECIUM AURELIA[10]

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Communicated August 10, 1937

Recently, Sonneborn[1] reported the discovery of a sexually differentiated race of *Paramecium aurelia*. In this race, *S*, every individual is of one of two sexes, called by him sex I and sex II; and the vegetative progeny of any individual are all of the same sex as the progenitor. When cultures of different sex are mixed, the individuals unite at once for conjugation; but when cultures of the same sex are mixed, no conjugation occurs. Sonneborn reported in detail the inheritance of sex at conjugation but gave only preliminary, qualitative results on its inheritance at endomixis. The present paper gives a precise and quantitative analysis of the segregation, inheritance and determination of sex at endomixis, and of certain related matters.

The inheritance of sex at endomixis was examined in three groups of individuals of the race *S* reported on by Sonneborn.[1] Each group consisted of a number of daily isolation lines descended vegetatively from a single individual. The three original individuals were closely related, but two of them were of sex I and one was of sex II. Samples from all the lines of the three groups were stained daily according to the method of Sonneborn[2] to determine when endomixis occurred. After each period of endomixis in each of the groups, all but one line was discarded; and a new set of lines was expanded from the one retained. To perpetuate the group a line was always selected of the same sex as the original progenitor. In this way, there were always maintained throughout the course of the work two groups of sex I and one group of sex II.

Segregation of Sex.—Sonneborn[1] found that some endomictic individuals give rise to two lines of descent differing in sex and that this segregation of sex occurs soon after the beginning of endomixis. The first question for analysis, therefore, was to ascertain the precise point at which this segregation occurs. As endomixis is a process which extends through several fissions, it was necessary to establish a definite point of reference for the study.

A brief description of the process will be necessary to make clear the subsequent account.

According to Woodruff and Erdmann,[3] endomixis begins with the gradual disintegration of the macronucleus. After the stage known as climax, there may or may not be a fission. In either case in each of the one or two individuals formed, two products of the micronuclei transform into new macronuclei through preliminary growth stages known as anlagen. These two anlagen are then segregated into separate individuals at the next fission, thus restoring the normal number of one macronucleus. In the subsequent fissions, the new macronuclei divide; and the fragments of the old macronucleus are irregularly segregated. Eventually the latter become resorbed, but they are visible in diminishing numbers for several fissions.

In order to ascertain the point at which sex is segregated, it was necessary to obtain and recognize those individuals in which had occurred the complete disintegration of the old macronucleus and the formation of the two anlagen of the new macronuclei. This was accomplished in the following way. Small mass cultures were set up consisting of the surplus animals from the isolation lines of the group to be tested. Endomixis was then induced by the method described by Sonneborn.[4] Observations were made at least every twelve hours to be sure that no conjugation had occurred. In no case was it ever found. At various intervals, a number of animals from the culture were stained with aceto-carmine to determine whether endomixis was occurring and if so, what stage the animals were in. When the culture was found to contain a large number of animals with two anlagen, some in earlier stages, some non-endomictics, and a very few or none with one anlage—i.e., after the first division after the formation of the anlagen—it was considered suitable for selecting animals in the two-anlagen stage. At this time, many of the animals with two anlagen are larger than most (or all) non-endomictics, and they may further be distinguished by their clear and narrow anterior end. Animals with these characteristics were chosen; and in every experiment some of those chosen, both before, during and after the selection of animals for the experiment, were stained in order to be sure that the selection was made accurately. That it was is shown by the fact that of 171 animals so chosen and stained 157 had two anlagen, 3 were in climax, 2 had only one anlage and 9 were non-endomictics. Those in the two-anlagen stage and probably those in climax were those required for the study. The non-endomictics were always eliminated by discarding those lines in which no fragments of the old macronucleus were found in one of the products of the second or third fissions. This method assures the selection of groups in which practically all the animals are in endomixis just prior to the first fission after the formation of the anlagen since, as the data show, not more than 3% of these groups were animals which were in endomixis prior to the formation of the anlagen or after the

first fission. This error is too small to appreciably affect the results.

The cultures to be tested for sex were obtained by allowing each of the selected endomictic individuals to multiply in from 4 to 5 drops of culture fluid until many animals were present and the food supply had begun to decline. Each culture was then tested for sex type by adding some of the individuals to each of two groups of animals: one group known to be sex I; the other, to be sex II. In nearly all cases conjugation occurred in one of these cultures only. The few exceptions to this are now under investigation. The sex of the animals tested was, of course, the opposite of that of the animals with which conjugation occurred.

Two extensive experiments were performed to determine the fission at which sex segregates at endomixis. In these experiments the two products of the first fissions of 181 individuals in the two-analgen stage of endomixis were isolated, and their progeny later tested for sex type. The two cultures from 96 of these individuals proved to be of the same sex, the two cultures from the remaining 85 individuals were of opposite sexes. Thus sex segregates at the first fission after the formation of the anlagen in approximately one-half the endomictic individuals.

In order to determine whether sex also segregates at a later fission, 353 products of the first fission after the formation of the anlagen were allowed to divide a second time; and, in each case, the two products of this second fission were isolated, and their progeny later tested for sex type. In 349 of the 353 cases, the two cultures thus produced proved to be of the same sex. In two of the other four cases, one of the cultures was of sex I; and the other formed pairs when mixed with animals of either sex. In the remaining two, one culture was of sex II; and the other reacted with both sexes. Three of the four exceptional cultures also contained conjugants at the time they were tested for sex type. None of these four cultures was tested for sex type sooner than six days after endomixis. Whether these four cultures actually represent exceptions to the general rule that the segregation of sex occurs only at the first fission after the formation of the anlagen or whether they can be explained on some other basis remains for a more thorough investigation to answer. It may therefore be concluded that sex never, or very rarely, segregates at a later fission than the first after the formation of the anlagen.

Sex Ratios.—The two sexes were produced in three different ratios in the six experiments that were performed. In four of the experiments the following numbers were obtained:

EXPERIMENT	1	2	3	4	TOTAL
Sex I	71	26	66	88	251
Sex II	63	21	55	94	233

These results are fairly close to the exact 1:1 ratio of:

EXPERIMENT	1	2	3	4	TOTAL
Sex I	67	$23^1/_2$	$60^1/_2$	91	242
Sex II	67	$23^1/_2$	$60^1/_2$	91	242

In another experiment 128 individuals of sex I and 63 of sex II were obtained, this being almost exactly a 2:1 ratio (mathematically, $127^1/_3 : 63^2/_3$). In a third experiment 63 individuals of sex I and 137 of sex II were obtained, which is close to a 1:2 ratio (mathematically, $66^2/_3 : 133^1/_3$).

Sonneborn found three different classes of endomictics: those that give rise solely to sex I; those that give rise to both sex I and sex II; and those that give rise solely to sex II. The same three classes were found in the present work. If random distribution of the two sexes to the two products of the first fission occurs, the proportion of each of these classes produced depends on the ratio in which the two sexes occur. With a 1:1 sex ratio, a 1:2:1 distribution of the three classes is expected; with a 2:1 sex ratio, a 4:4:1 distribution; and with a 1:2 sex ratio, a 1:4:4 distribution. In the four experiments which gave a 1:1 sex ratio, the following numbers of endomictics of the three classes were obtained:

EXPERIMENT	1	2	3	4	TOTAL
All sex I	19	5	14	21	59
Sex I and sex II	31	11	31	45	118
All sex II	13	3	10	22	48

With an exact 1:2:1 ratio, the following would be the numbers:

EXPERIMENT	1	2	3	4	TOTAL
All sex I	$15^3/_4$	$4^3/_4$	$13^3/_4$	22	$56^1/_4$
Sex I and sex II	$31^1/_2$	$9^1/_2$	$27^1/_2$	44	$112^1/_2$
All sex II	$15^3/_4$	$4^3/_4$	$13^3/_4$	22	$56^1/_4$

In the experiments which gave a 2:1 sex ratio, the numbers of endomictics of each of the three classes were 42:40:11, almost identical with the theoretical numbers ($41^1/_3 : 41^1/_3 : 10^1/_3$). In a third experiment, in which a 1:2 sex ratio was obtained, the number of endomictics of each of the three classes were 13:36:46 which is fairly close to the theoretical 1:4:4 ratio of $10^5/_9 : 42^2/_9 : 42^2/_9$. Therefore it can be concluded that the distribution of the two sexes to the products of the first fission after the formation of the anlagen is purely random.

Change of the Sex Ratio in a Single Line of Descent.—In two of the three groups examined, a different sex ratio was obtained in the second test from that obtained in the first. In one, the ratio changed from 1:1 to 2:1; in the other from 1:2 to 1:1. The two groups had a similar history between the tests. Each went through many vegetative fissions; each went through endomixis, the first once, the second twice; but neither went through con-

jugation. Therefore the change of ratio must have occurred either at vegetative fission or at endomixis, though neither of these processes necessarily involves a change of ratio since the third group went through both without changing its ratio. However, change of ratio must occur with considerable frequency since it was found in two out of the three groups twice tested.

Sex Determination.—The relations reported here for endomixis and those reported by Sonneborn[1] for conjugation are very similar. In both processes sex is segregated to different individuals at the first fission after the formation of the anlagen; and, at this same fission in both, the two new macronuclei that are formed are also segregated. Thus it is extremely probable that sex is determined by the macronucleus and that two different types of macronuclei can be formed: one determining sex I; and one determining sex II. Thus far sex determination at endomixis is just like that reported by Sonneborn for conjugation in this race. However, the experiments reported here show that after endomixis there are at least three sex ratios while Sonneborn reports only one, the 1:1 ratio after conjugation.

Sonneborn has suggested two possible chromosome mechanisms to account for the facts that he observed. These apply equally well to endomixis when a 1:1 ratio occurs. However, modifications are needed to explain the 2:1 and 1:2 ratios and also to explain the change of ratio. What the chromosome mechanism actually is can only be determined after a more thorough study.

Relation of the Results to Other Problems.—The results reported here have a bearing on several other problems of protozoölogy.

(1) It has been held in some quarters that endomixis is a variable reaction to diverse environmental factors and not a definite process as is conjugation. The preciseness of the ratios and of the segregation of sex as shown by these experiments indicates, on the other hand, that endomixis is quite as regular and exact a process as is conjugation.

(2) Diller[5] contends that much of what has been called endomixis is in reality autogamy or self-fertilization. While perhaps the close similarity of sex inheritance at conjugation and at endomixis seems to indicate that this is the case, actually the present results neither confirm nor contradict this contention but could equally well be explained on the basis of endomixis as described by Woodruff and Erdmann[3] or on the basis of autogamy as described by Diller.[5]

(3) The origin at endomixis of genetic diversities in other characters than sex has been reported by Erdmann,[6] Parker,[7] Caldwell[8] and Sonneborn and Lynch.[9] The fact that within a clone the macronuclei of different products of the first division after the formation of the anlagen at endomixis may differ in their sexual potency makes it seem possible that they may also differ in their potency in relation to other characters, thus explaining to a

degree the origin of hereditary diversities at endomixis. To what extent, if at all, this may be true remains for future investigation to discover.

[1] Sonneborn, T. M., *Proc. Nat. Acad. Sci.*, **23**, 378–385 (1937).
[2] Sonneborn, T. M., *Genetics*, **21**, 503–514 (1936).
[3] Woodruff, L. L., and Erdmann, R., *Jour. Exptl. Zoöl.*, **17**, 425–518 (1914).
[4] Sonneborn, T. M., *Biol. Bull.*, **72**, 196–202 (1937).
[5] Diller, W. F., *Jour. Morph.*, **59**, 11–67 (1936).
[6] Erdmann, R., *Arch. f. Entwmech.*, **46**, 85–148 (1920).
[7] Parker, R. C., *Jour. Exptl. Zoöl.*, **49**, 401–439 (1927).
[8] Caldwell, L., *Jour. Exptl. Zoöl.*, **66**, 371–407 (1933).
[9] Sonneborn, T. M., and Lynch, R. S., *Genetics*, **22**, 284–296 (1937).
[10] The author wishes to acknowledge his great indebtedness to Dr. T. M. Sonneborn for his constant interest and many valuable suggestions throughout the course of the work.

Reprinted from GENETICS 24: 49–58, January, 1939

A DELAYED CHANGE OF PHENOTYPE FOLLOWING A CHANGE OF GENOTYPE IN PARAMECIUM AURELIA

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Received September 22, 1938

INTRODUCTION

THE present investigation, dealing with a delayed change of phenotype following a change of genotype, was performed on the stock S of the ciliate protozoan *Paramecium aurelia*. This stock is known (SONNEBORN 1937) to consist of two classes of individuals. When animals of one class are mixed with those of the other, they immediately form clumps and soon pair off for mating; but when animals of the same class are mixed, no such clumping and pairing occurs. As the conjugant pairs that form always consist of one animal of each class, the classes (originally called sexes I and II) have been designated mating types I and II (SONNEBORN 1938).

So long as nuclear reorganization does not occur, all the vegetative progeny of a single individual of either type are ordinarily of the same type as that individual; but after such a reorganization the mating types are redetermined in definite ratios. It thus happens that following nuclear reorganization some lines of descent are of the same mating type as before and some are now of different type. These relations are set forth in the papers of SONNEBORN (1937) and KIMBALL (1937). In the present paper, it will be shown that in the case of the change of genotype from type II before nuclear reorganization to type I afterwards, at least some animals remain phenotypically type II for a short time after the change. This is shown by the fact that at this time some of the animals give the mating reaction when mixed with type I. It will further be shown that the change of phenotype occurs at different rates in different individuals of the same culture, for at the same time that some react with type I others react with type II. Thus as both type I and type II animals are present in the same culture, conjugation can and does occur between them. In such cases, however, all the vegetative progeny are later exclusively of type I, even the descendants of those that were type II at an earlier stage. This shows that all animals were, from the first, genotypically type I even when they behaved, during a brief period, as type II. These phenomena are obviously of importance in relation to the problem of the interaction of the genes and the cytoplasm.

[1] The writer is deeply indebted to Dr. T. M. Sonneborn for the interest that he has taken in the work and for his many valuable suggestions concerning it.

In the present work, attention was confined to the changes occurring at the nuclear reorganization process, commonly known as endomixis, that occurs periodically within single unmated individuals. The essential features of this process for present purposes are the disintegration and eventual disappearance of the old macronucleus and the derivation of two new macronuclei from the micronuclei. Of these two macronuclei, one passes undivided to each of the daughter cells formed at the first fission. At subsequent fissions, the new macronuclei divide normally; and the fragments of the old macronucleus gradually disappear though they persist in diminishing numbers for several fissions. All the vegetative progeny of an individual containing only one new macronucleus are, ordinarily, genetically alike in mating type. Such a group of individuals is known as a "caryonide" (SONNEBORN 1938).

MATERIAL AND METHODS

To obtain material for the experiments, it was necessary to start out with caryonides of type II, induce endomixis in them, isolate the new caryonides, and ascertain which had changed genotypically to type I. This was done in the following way. Caryonide cultures were tested against standard cultures of the two mating types. Those that conjugated with type I but not with type II were of type II and suitable for further work. Endomixis was induced in such cultures according to the method of SONNEBORN (1937a); and individuals in endomixis were isolated. After the first fission of each isolated animal, one daughter cell was stained and the other kept alive. If the stained animal was in endomixis and contained not more than one new macronucleus, the living sister animal was retained as the progenitor of a caryonide; otherwise it was discarded. The new caryonides were all cultivated as daily isolation lines; that is, each day one animal was transferred from the previous day's culture to two drops of fresh culture medium in a fresh depression slide. The animals left over after the isolations of the first two or more days were kept without addition of culture medium for further study.

Not all these caryonides, however, were studied in detail; for, since many of them were type II and thus not suitable for study, this would have involved much unnecessary labor. Detailed studies were made only on those caryonides in which conjugants appeared in the first or second left-over cultures. The appearance of conjugants, suggesting the presence at this time of animals of both mating types in cultures presumably pure for one genotype, marked out these caryonides for the study of delayed changes of phenotype. Therefore, all other carynonides were discarded. Observations for conjugation were made on later left-over cultures of the caryonides retained in order to ascertain whether the occurrence of both

types was a transient or permanent characteristic. Tests for mating type were also made on one or more left-over cultures (but not the first one) in order to ascertain whether these caryonides were actually genotypically type I. All of the caryonides dealt with in this paper were genotypically type I. However, a few whose behavior was very different from the rest are to be reported upon elsewhere.

This method of selecting the material involved as an essential the detection of conjugation in the left-over cultures as quickly as possible. As decrease in the available food is necessary for conjugation to occur, the left-over cultures were made with as little of the nutrient culture fluid as practicable (2 drops). Observations for conjugation were made on these cultures at not greater than eight hour intervals from the time that 8 to 16 animals were present until the cultures were completely starved and there was no further likelihood of conjugation occurring. These observations were invariably made on the first two left-over cultures and frequently also on later ones.

It was essential, furthermore, to be sure that the conjugation which occurred was not the consequence of a new endomixis which would produce animals of both mating types. Therefore, samples of the isolation lines and left-over cultures were stained and examined frequently. In a few rare cases, endomixis was found in the first few days after the previous endomixis; but otherwise it was not found until 10 days after it. All caryonides in which very early endomixis was found were discarded.

THE EARLY PRESENCE OF BOTH MATING TYPES

Of the 141 available caryonides that had been changed by endomixis from type II to type I,[2] 21 were tested for the presence of both mating types in the first left-over culture. To do this it was desirable to pick out for testing animals of each mating type if such existed in these cultures. Therefore advantage was taken of the occurrence of conjugation; for presumably when two individuals come together for conjugation, they are of different mating types. This matter was put to a direct test by isolating pairs that had just come together for mating but were not yet firmly united. These were then separated by drawing them in and out of a pipette a few times, and the two animals thus separated (called hereafter a "split pair") were individually tested for mating type. The tests were carried out

[2] This total of 141 caryonides includes the 17 found in one experiment in which there appears to have been an error in labelling. These 17, according to the records, came from a single culture, marked type I, in which endomixis had been induced. In the same petri dish was another culture marked type II. The caryonides isolated from these two cultures showed by their subsequent behavior just the reverse of what would have been expected from the labels. Hence, it is assumed that the labels were inadvertently reversed and that the 17 caryonides in question actually came from a culture which, before endomixis, was type II instead of type I.

as shown in figure 1. Each member of a split pair was placed separately with a group of animals of known mating type, in some cases type I and in some cases type II; and observations for mating reactions were made. The animals of known mating type were always considerably larger in size than the animals to be tested so that in all cases it was possible to identify the latter. In every case in this first part of the test, only one member of the split pair gave the mating reaction. After about one-half to

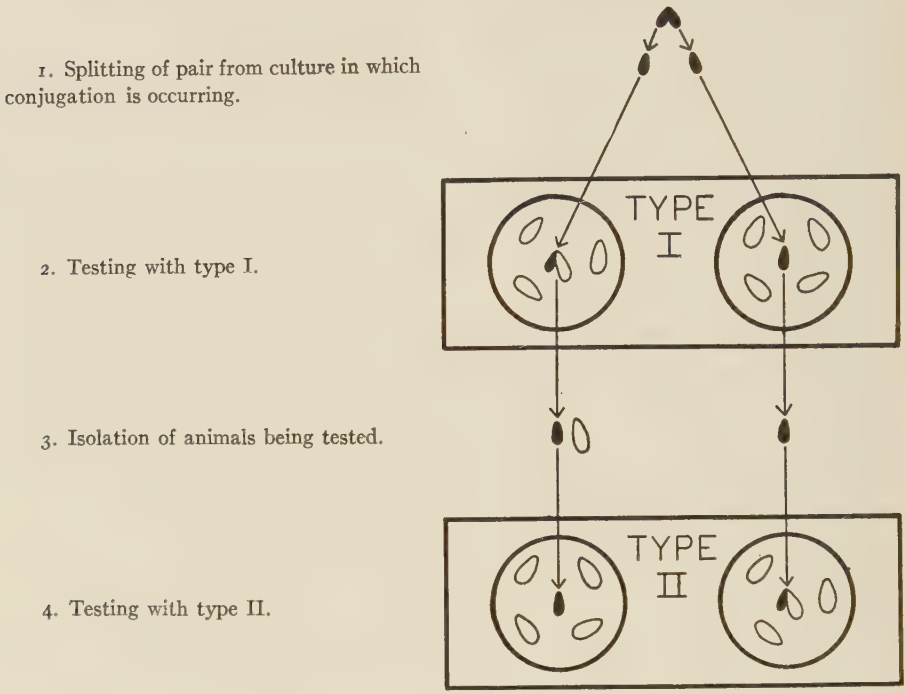
1. Splitting of pair from culture in which conjugation is occurring.
2. Testing with type I.
3. Isolation of animals being tested.
4. Testing with type II.
TYPE I
TYPE II

FIGURE 1.—Diagram showing the method used to test the two members of a split pair individually for mating type. The animals shown in solid black are those being tested for mating type. Those shown in outline are the animals of known mating type used for the testing.

three-quarters of an hour, the members of the original split pair as identified by their smaller size were withdrawn and placed with large animals of the other mating type. Again not more than one member of the original split pair reacted and this time it was the one that had not reacted in the first part of the test. Thus one member of the original split pair reacted with type I but not with type II; and the other member with type II but not type I. Hence, one member of the original pair was of type I and the other of type II.

The complete demonstration that conjugation in these cultures occurred between type I and type II animals was made for 16 of the 22 pairs tested.

In the other 6 pairs, only one member of each pair reacted. This one, however, reacted with type I alone; and so it was type II. Nevertheless, as will be shown later, this animal as well as the other one gave rise, when they were allowed to multiply, to type I animals only. Several other pairs, not recorded here since they yield no information on the question in hand, either entirely failed to react; or, if one member of the pair did react, it did so as type I. Why some of the animals did not react is not certain.

The foregoing analysis showed not only that the 21 caryonides whose split pairs were tested contained both types I and II but also that the occurrence of conjugation within a culture is evidence that both types are present, for conjugation has been shown to occur only between these two types. Hence, the remaining 120 caryonides for which no split pairs were individually tested must have contained animals of both types for conjugation occurred in them also.

THE PRESENCE OF BOTH TYPES A TRANSIENT PHENOMENON

Further study demonstrates that the presence of both types in these caryonides is a transient phenomenon; for conjugation, which is evidence for the presence of both types, occurred only in the early left-over cultures of these caryonides. Conjugants were found only in the first left-over culture in the case of 124 caryonides, including the 21 whose split pairs were tested individually for mating type. They were found in the first two left-over cultures but in no later ones in the case of 13 caryonides and in only the second left-over culture in the case of 4 caryonides. No pairs were found in any later cultures. In this study, two successive cultures were examined for 32 caryonides, three for 99, and four for 10.

GENETIC MATING TYPE OF THE CARYONIDES

It is clear from the evidence that even the type II animals of the first left-over cultures must have been genetically type I. In every case, at least one left-over culture, but not the first, was tested for mating type. Every one of the 141 caryonides dealt with here reacted in these tests solely as type I. Thirteen caryonides, not otherwise included in the discussion, though they reacted for the most part as type I did give a weak reaction as type II in at least one test. Eight of these were found in one experiment in which there was cause to suspect that the standard cultures used for the first two tests were not pure for one mating type. A fourth left-over culture of 6 of these 8 caryonides was tested with fresh standard cultures which were certainly pure for one mating type; and, in every case, this last culture reacted solely as type I. It is not impossible that the standard cultures used to test the other five caryonides were likewise impure. It seems probable, then, that the reason some of the left-over cultures of each

of these thirteen caryonides reacted with both standard cultures was that the standard cultures themselves contained animals of both types.

More direct evidence that all animals of the 141 caryonides with which this paper for the most part deals were genotypically type I is furnished by a study of cultures derived from the members of split pairs. Animals which had recently come together for conjugation were split and each animal isolated separately. In the case of the 22 pairs whose members were tested individually for mating type, the animals were isolated after the testing had been completed. In all cases, these isolated animals were allowed to divide once; and one of the two sister animals was stained and examined for signs of nuclear reorganization. In the cases considered here, none was found; and the living sister animal was allowed to give rise to a culture which was then tested for mating type. In 52 pairs of the 54 so tested, including the 22 which had been shown by individual tests to consist, when mating, of two animals of opposite type, the cultures from the two members of a pair both reacted as type I only. The two exceptional pairs will be discussed in the next section. Since all individuals finally give rise only to type I animals, it seems clear that they are all genotypically type I.

RATE OF CHANGE OF PHENOTYPE

The rate of change from type II to type I is to some extent variable. This is, of course, true for a single caryonide since, in the first left-over cultures, animals of both mating types are present at the same time. It was also found that some caryonides as a whole completed the change in a shorter or longer time than usual. Thus a number of caryonides were found which, although changed by endomixis from type II to type I, gave rise to no left-over cultures containing conjugants. While conjugation may have been overlooked in a few of these, it seems probable that in most of them the change occurred so rapidly that no type II animals were present by the time the first left-over culture was ready to conjugate.

In other cases, the change appears to have taken a longer time than usual. Thus, as already mentioned, 17 of the caryonides gave rise to a second left-over culture which contained conjugants. Moreover, two other caryonides appear to represent cases of such slow change. Only the first left-over culture of each of these caryonides contained conjugants. A later left-over culture from the isolation line of each reacted solely as type I. In each case, one split pair was obtained from the first left-over culture, and cultures were raised from the two members. In both cases, the culture from one member of the pair reacted solely as type I while the culture from the other member reacted with both types but more strongly as type I. Although the behavior of these two caryonides could be explained in other ways, it seems likely that all the descendants of the type II members of

these split pairs had not yet changed to type I by the time the cultures from them were ready to test. This cannot be demonstrated, however, since no other cultures were obtained from these split pairs.

DISCUSSION

When at endomixis, the genotype changes from type II to type I, there appears to be a lag in the corresponding change of the phenotype. In the first, and occasionally the second, left-over cultures derived from the daily isolation lines of these caryonides, there were found to be not only some animals which had changed to type I but also others which were still type II. However, these type II animals gave rise only to cultures which were composed entirely of type I animals. Therefore, the caryonides were genetically type I; and the presence of type II individuals in the early left-over cultures is in all probability due to a lag in the change of the old phenotype.

Such a lag in the change of phenotype following changes of genotype has been reported previously for Paramecium by SONNEBORN and LYNCH (1934) and by DEGARIS (1935). These investigators studied this matter in connection with changes of genotype occurring at conjugation. The two members of a pair of conjugants were found to be alike in genotype following conjugation. However, if the two members of a pair differ in their characteristics before conjugation, it was found that for a time after conjugation they likewise differ but that they gradually approach the common characteristics determined by their like genotypes.

SONNEBORN and LYNCH (1934) working on *Paramecium aurelia* reported such delayed changes for the characters, fission rate and body length. They found that the whole process took from 5 to 14 days in the case of fission rate and from 5 to 8 days in that of body length. Much as in the case reported here, the rate of change varied from clone to clone and was not always the same for two lines of a single clone.

DEGARIS (1935) reported similar phenomena in *Paramecium caudatum* with the character body length. However, he found that the lag period was either lacking or, in some cases, present but very brief for the character fission rate.

Similarly in the present work, no lag period could be detected for a number of caryonides which had changed from type II to type I; and none was ever found for the change from type I to type II. It must be remembered that there was a period of two or three days between endomixis and the time when the first left-over culture was ready to conjugate during which it has not been possible to make studies of mating type. The change of phenotype, then, would not have to be instantaneous in order that no lag in it be found.

Such delayed changes of phenotype have also been reported for Metazoa. TOYAMA (1913) reported that in the silkworm moth, *Bombyx mori*, the color of the egg is determined in some cases by the genetic constitution of the mother although the structure that is colored (the serosa) has the new hybrid genetic constitution.

Both BOYCOTT and DIVER (1923) and STURTEVANT (1923) have interpreted the former's data to show that in the snail *Limnea peregra*, the direction of coiling of the shell is dependent on the genetic constitution of the mother. DIVER, BOYCOTT, and GARSTANG (1925) on the basis of further evidence confirm this interpretation although they show that certain complicating factors are involved.

REDFIELD (1926) reports a lethal effect in *Drosophila melanogaster* that is determined by the genetic constitution of the mother. This lethal kills in the egg stage a considerable part of the female progeny of a female homozygous for the gene involved but has no appreciable effect on the male progeny. REDFIELD thinks that this maternal effect may be due either to an effect on the egg of its unreduced chromosome constitution or to an indirect influence impressed on the egg from without by the mother either before or after reduction. If this last possibility is the correct one, this would not be a case of a lag in the change of phenotype. However, if the first is correct, it would clearly be one.

SEXTON and PANTIN (1927) report a clear case of such a lag in phenotypic change in the crustacean, *Gammarus chevreuxi*. They found that when a female homozygous for the mutant gene "white," was crossed to a male carrying the gene "new red," all the offspring were at first white but later all or half of them become red in color depending on whether the "new red" male was homo- or heterozygous. Similar cases were found with certain other mutant genes.

STURTEVANT (1929) reported that homozygous claret females of *Drosophila simulans* produced a large percent of gynandromorphs and other abnormalities due to irregularities in chromosome behavior both at maturation and during early cleavage. Claret males and heterozygous claret males and females produce no such large percent of abnormal offspring. It thus appears that the effects of the homozygous claret condition in the unreduced eggs last over into the early cleavage stages.

Thus it appears that the occurrence of a time lag in the change of phenotype following changes of genotype is a widespread phenomenon. Cases have been found in such distantly related groups as the Protozoa, the Mollusca, and the Arthropoda.

SONNEBORN and LYNCH (1934) have pointed out two factors that might be the physical basis of the delayed change in phenotype in Paramecium: continued activity of the old macronuclear fragments and delayed change

of the old cytoplasm under the action of the new genotypic constitution. While DeGaris (1935) does not go into this matter in as much detail as Sonneborn and Lynch, he does suggest that the cytoplasm is involved perhaps under the influence of the old macronuclear fragments. The present evidence does not furnish any basis for a decision between the two alternatives.

SUMMARY

1. In the ciliate protozoan *Paramecium aurelia*, a lag in the change of phenotype following a change of genotype from mating type II to mating type I was found after endomixis.

2. In the early left-over cultures of caryonides that had been changed from type II to type I, conjugation was found; and it was shown that type II as well as type I animals were present.

3. Later left-over cultures, however, contained only type I animals.

4. These caryonides were clearly genetically type I since not only type I but also type II individuals from the early left-over cultures gave rise only to type I animals.

5. The rate of the change of phenotype varied for different caryonides and for different animals of the same caryonide.

6. No such lag was found in cases of change from type I to type II and also not in some cases of change from type II to type I, possibly because it was too short to be detected.

7. Similar cases of a lag in the change of phenotype have been found following genetic changes at conjugation in Paramecium. Certain types of maternal effects in Metazoa seem to be the same sort of phenomenon.

8. The physical basis of the lag in the change of phenotype in Paramecium could be either a continued activity of the old macronuclear fragments or a delay in the change of the old cytoplasm under the action of the new genotype.

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Reprinted from THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 81, No. 2, July, 1939

CHANGE OF MATING TYPE DURING VEGETATIVE REPRODUCTION IN PARAMECIUM AURELIA

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The stock S of the ciliate protozoan Paramecium aurelia has been shown by Sonneborn ('37) to consist of two types of individuals called by him (Sonneborn, '38) mating types I and II. Conjugation occurs only between individuals of opposite types, never between those of the same type. Sonneborn found that the vegetative progeny of a single individual are all of the same mating type as long as no nuclear reorganization (endomixis) occurs, and Kimball ('37) confirmed this as the usual situation but found certain exceptions that required further investigation. The exceptions have turned out to be of two main kinds. One of these has already been reported (Kimball, '39). It is confined to caryonides (the group of vegetative descendants of an individual containing one macronucleus) genetically of type I that arose at the previous endomixis from type II animals and, in these, is confined to individuals produced during the first few days after endomixis. Some of these individuals are for a short time phenotypically type II and then change to type I without any further endomixis. This behavior, then, must be due to a lag in the change of the phenotypic mating type following a change in the genotypic mating type. The other kind of change of mating type during vegetative reproduction is to be described in this paper. It differs from the first kind in four ways: 1) It is extremely rare, occurring in only about 1 to 3% of the caryonides arising at endomixis. 2) It is not

[1] The writer wishes to express his deep appreciation to Dr. T. M. Sonneborn for the continual interest that he has shown in the work and for the many valuable suggestions that he has made concerning it.

restricted to caryonides descended from individuals that were type II before the last endomixis but occurs with approximately the same frequency among the caryonides descended from those that were type I. 3) In any one line of descent change of mating type occurs not only once but repeatedly. 4) Change of mating type occurs not merely during a restricted period of time but often throughout the entire period of existence of the caryonide. Because of the latter two characteristics, the caryonides exhibiting this behavior were designated 'unstable.' The following account is based on a study of sixteen such unstable caryonides. In the first section, proof is given that in these unstable caryonides both mating types are continually being produced during vegetative reproduction. In the following sections, evidence is presented on the problems of the direction and frequency of the changes of type; on the occurrence of occasional stability of mating type within these caryonides; and on inheritance following endomixis in unstable caryonides. Finally, the questions of the determination of mating type in unstable caryonides and of the relation of the phenomena set forth here to other work in protozoan genetics are discussed.

THE REPEATED PRODUCTION OF BOTH MATING TYPES DURING VEGETATIVE REPRODUCTION IN UNSTABLE CARYONIDES

The sixteen unstable caryonides that form the subject of this paper were found by looking for caryonides (the methods used for obtaining caryonides and cultures from them are given by Kimball, '39) in which successive mass cultures contained conjugants although each culture was derived from a single animal. The occurrence of conjugation suggests, although it does not prove, that animals of both mating types were present in these cultures. In fifteen of the sixteen unstable caryonides, conjugants were found in four to eight successive cultures. In the remaining caryonide only three cultures contained conjugants. This, however, is considered to belong to the same category for reasons that will be set forth later.

Since the existence of a caryonide is limited to the time between two successive endomixes and since endomixis recurs 8 to 12 days after the origin of the caryonide, it is ordinarily not possible to obtain more than five to eight successive cultures for observation. Hence, the preceding data show that the occurrence of conjugation in cultures of these caryonides continues, at least in some caryonides, throughout their entire history. It is, moreover, not merely a long lasting but also a widespread feature, as shown in several caryonides by the occurrence of conjugation in a considerable number of all the mass cultures studied. Thus 48 out of 75 cultures of one caryonide contained conjugants, 30 out of 50 of another, and 32 out of 73 of another. For each of these caryonides, several isolation lines were maintained; and from each of these lines mass cultures were obtained daily thus allowing the examination of a large number of cultures. However, all save one of the unstable caryonides gave rise to at least one culture which contained no pairs, and in some cases these were by far the most numerous kind of culture. These cultures containing no pairs will be dealt with in detail in a later section.

The evidence just presented shows clearly that caryonides exist in which the vegetative progeny of single individuals conjugate. This suggests that animals of both mating types were produced among the vegetative progeny of a single animal. To show that this is so, it was necessary to demonstrate directly that conjugation within these caryonides occurs only between animals of different mating type. This was done in much the same way as set forth in a previous paper (see fig. 1, Kimball, '39). Pairs of animals that had come together for mating in cultures of these caryonides were separated before they were firmly united. The two members of these 'split pairs' were placed separately, first with animals known to be of one mating type and then with those known to be of the other. During this testing, the members of the original pair were distinguished from the test animals by their considerably smaller size. In any case

in which this distinction was not clear, the pair was discarded. About half the split pairs were tested with type I first and half with type II first. In all cases, any one individual reacted only with one mating type and not with the other. In every case in which both members of the split pair reacted, one member reacted with one mating type and one with the other. In some cases, the member of the split pair which reacted in the first part of the test and not in the second was put back with animals of the mating type with which it had previously reacted. Frequently it reacted once more. This demonstrates that these animals had not, for any reason, lost their ability to react during the intervening period when they had not reacted. Their failure to react during this period must have been due to the fact that they had been with animals of the same mating type as themselves.

In all, thirty-eight pairs from sixteen cultures of four-caryonides were tested in this manner. The complete test, exactly as described, was obtained with fifteen of these pairs. All save the last step was obtained with ten others. In thirteen other split pairs, only one member reacted; the other did not react with either mating type. In the case of three of these thirteen, the member which did react was tested twice with one mating type, reacting both times, but not reacting during an intermediate period with animals of the other mating type. A few of the animals which did not react had obviously been injured during the process of splitting the pairs from which they were derived. Why the rest did not react is not, at present, clear.

The occurrence of conjugation in cultures of these caryonides, then, appears to indicate the presence of animals of both mating types. Since each of these cultures was derived from a single individual, the repeated occurrence of conjugation in these caryonides indicates that repeated changes of mating type are occurring.

It is known (Kimball, '37) that such changes of mating type in the progeny of a single individual occur regularly at endomixis. It is, therefore, important to ascertain whether

endomixis is the cause of the frequent changes of mating type observed in these unstable caryonides. This is obviously improbable because the intervals between successive endomixes in this stock are rarely less than 10 days and the changes of mating type described above were occurring repeatedly between the third and the ninth days after the endomixes at which the caryonides arose. Exceptional cases of endomixis recurring within such short intervals are known, however, so that it was necessary to ascertain directly whether this was happening in these caryonides.

The method of investigating this question made use of three well-known facts: that endomixis requires for its completion the passage of several successive fissions; that it can be detected throughout these fissions in stained preparations by the presence of fragments of the old disintegrated macronucleus; and that during at least one stage of endomixis there is a marked lowering of the fission rate. Accordingly, the method employed was to allow a single animal to go through several fissions and to start small mass cultures from many of the products of these fissions. Examination was made for endomixis by staining products of fissions at short intervals, and close watch was kept for the significant drop in fission rate. In no case reported here, was there found either a slowing of fission rate or animals containing macronuclear fragments. The small mass cultures obtained in this way were observed for conjugation and tested for mating type.

In experiments 1, 2 and 3 after the first fission of the animal selected to start the experiment, one of the two animals produced was stained. The other was allowed to divide twice, and the four products of this division were isolated. One product of the next division of each was then stained. The unstained animals were each allowed to divide twice again, and the sixteen animals produced were isolated. Again one product of the next fission of each was stained. In no case, were any animals with macronuclear fragments found. Hence, it is certain that no endomixis occurred during the derivation of the sixteen animals from the original one. Each

of these sixteen animals was then placed in a single drop of culture fluid, and after four or five fissions (during a period of about a day and a half) their progeny were ready to conjugate and were tested for mating type. The results of these tests were as follows:

Experiment 1. Of the sixteen cultures tested, four reacted exclusively as type II while the remaining twelve cultures reacted with animals of both types. Of the latter, two contained more animals of type I than of type II, eight contained animals of both types in about equal numbers, and two contained more animals of types II than of type I.

Experiment 2. Of the sixteen cultures, five were not tested. Of the remaining eleven, three reacted exclusively as type II and eight contained animals of both types. Of the latter, four contained more animals of type I than of type II, two contained both types in about equal numbers, and two contained more animals of type II than of type I.

Experiment 3. Of the sixteen cultures, four were not tested. Of the remaining twelve, eleven reacted exclusively as type II and one reacted exclusively as type I.

In experiments 4 and 5, a slightly different method was used. After the first fission, one of the two resulting animals was stained. Four fissions then took place without staining; but as these all occurred within 24 hours, it is certain that time for endomixis to occur was lacking. Each of the resulting sixteen animals was isolated and one product of the next fission of each was stained. As macronuclear fragments were not found in any of these, endomixis was not and had not been in progress. The surviving sixteen animals were isolated in 1 drop of culture medium each, as in the previous experiments; and the cultures derived from them were tested after a day and a half during which about four or five fissions had occurred. The results follow:

Experiment 4. Of the sixteen cultures, two were not tested. Of the remaining fourteen, thirteen reacted exclusively as type II; and one, exclusively as type I.

Experiment 5. Of the sixteen cultures, one was not tested. Of the remaining fifteen, fourteen reacted exclusively as type II; and one contained animals of both mating types.

Thus in these last two experiments, as in the first three, animals of both mating types were produced among the progeny of a single individual in the absence of endomixis. There can be no question of the validity of this conclusion in the case of experiments 3 and 4 where the change of type must have occurred during the period of staining and expansion from a single individual. In the remaining experiments, the change of type took place during the development of the small mass cultures. However, it is equally certain that endomixis could not have been responsible for the changes of type in these; for, if it had occurred in the early stages of the cultures, there would have been a drop in fission rate; and this did not happen. If it had occurred later in the history of the cultures, the animals would still have been in endomixis when tested and so would not have reacted; for Sonneborn ('37) found that mating reactions do not occur during endomixis. It is, therefore, certain that changes of mating type in unstable caryonides occur in the absence of endomixis, during purely vegetative reproduction.

DIRECTION AND FREQUENCY OF CHANGES OF MATING TYPE IN THE UNSTABLE CARYONIDES

Direction of change of type. In a preceding paper (Kimball, '39), it has been shown that certain changes of mating type during vegetative reproduction occur in one direction only (from type II to type I) and are not reversible. In the unstable caryonides, on the other hand, the changes of type can occur in either direction and are reversible. This was shown by removing from cultures of unstable caryonides pairs of animals that had come together for conjugation but had not yet become firmly united. These were separated by drawing them in and out of a pipette a few times, and a small mass culture was allowed to develop from each. To be certain that the members of the 'split pairs' had not conjugated or

gone through endomixis, one product of the first fission of each member was stained and examined. No sign of nuclear reorganization was found in any of the cases reported here. In ten of the split pairs studied in this way, both members of the pair gave rise to cultures that contained individuals of both mating types. As it was shown on pages 3 and 4 that the members of a split pair are of different mating types and, as just shown, each may give rise to both types, it is clear that individuals of type I may produce descendants of type II and individuals of type II may produce descendants of type I. One of these ten split pairs was derived from the caryonide which was mentioned on page 2 as giving rise to but three cultures containing conjugants. Since changes of mating type occurred in both directions in this caryonide, it must have been an unstable one.

It is also clear that these changes of mating type are reversible. Since the two members of each of the ten pairs mentioned above were derived from a single animal, one of the two must have been changed in mating type. Since this changed animal in turn gave rise to animals of both types, the change must have been reversible.

Frequency of the change of type. The frequency of change of mating type during vegetative reproduction of unstable caryonides is probably variable but, at least in some cases, is not very high. It may, however, occur repeatedly among the vegetative progeny of a single animal. Thus, in some cases, a number of mass cultures obtained on successive days from one isolation line contained conjugants. Two such lines gave rise to eight successive cultures containing conjugants; two, to seven; six, to six; and eleven, to five. In other cases, a number of cultures, obtained simultaneously by expansion from a single animal, contained conjugants. In one case, all of the twelve cultures derived from an animal contained conjugants; in two cases, nine out of the twelve; and in one case, thirteen out of the sixteen. This repeated conjugation does not necessarily indicate a high frequency of change in these lines. One change occurring at a fairly early stage in the de-

velopment of each of these cultures would result in the production by the time the culture was ready to conjugate of a number of animals of the changed type. Of course, a higher rate of change than one per culture is not excluded and is, perhaps, not unlikely.

That, at least in some cases, the rate of change is low is indicated by the fact that a number of single and occasionally several successive cultures contained no conjugants. Thus each of eighteen lines gave rise to one culture; each of two lines, to two; and one line, to three successive cultures containing no conjugants. All these lines gave rise to at least one later culture which contained conjugants. Other lines were found which gave rise to larger numbers of cultures which contained no conjugants and to none that did. There are reasons for believeing that these lines are somehow different from the rest, and so they will be discussed in detail later.

Since tests for mating type were not made on the cultures of the three lines which gave rise to more than one successive culture containing conjugants, it cannot be said that the single animals that gave rise to each of the successive cultures did not undergo a change of mating type. Even if such changes did occur, the frequency of change in these lines could not have been greater than one change per culture. Since about 110 animals are involved at one time or another in the production of each culture, no more than one change could have occurred among 110 animals. Because of this very low frequency of change, it can be concluded that it is highly improbable that the mating type of each individual animal is redetermined at random.

Supporting evidence that the mating type is not redetermined at random for each individual animal is furnished by the study of cultures derived from animals of whose mating type had been ascertained by individual tests (see pages 3 and 4). Some of these animals were isolated after the testing and allowed to give rise to cultures. The six of these animals which were known to be of type I all gave rise to cultures which were composed entirely or for the most part of type I

animals. Of the eight animals which were known to be type II, five gave rise to cultures which were composed entirely or for the most part of type II animals; one, to a culture which was composed of about equal numbers of animals of each mating type; and two, to cultures which were composed for the most part of type I animals. Though the numbers are small, the fact that eleven of these fourteen animals produced mostly animals of the same type as themselves supports the conclusion that the mating type of each animal is not redetermined at random.

As mentioned above, a few lines derived from unstable caryonides were found which, apparently, were composed entirely of animals of one mating type and in which no change of mating type occurred. All six of these lines together with their sublines gave rise to a number of cultures containing no conjugants and to none which contained them. Three caryonides that were produced by endomixis from type I animals gave rise to four of these lines. The first of these caryonides gave rise to one line from which forty-two cultures containing no conjugants were derived. All these cultures reacted solely as type I. The second caryonide gave rise to two lines one of which produced twenty-six and the other four cultures containing no conjugants. Five of the cultures of the first line and all four of the second were tested for mating type and reacted solely as type I. The third caryonide gave rise to one line which produced twenty-six cultures, all containing no conjugants. Five of these were tested and reacted solely as type I. A fourth caryonide, produced by endomixis from type II animals, gave rise to two lines each of which produced eight cultures, all containing no conjugants. All sixteen of these cultures reacted, when tested, solely as type II.

It is clear that all four of these were unstable caryonides. All save the first gave rise to a considerable number of cultures containing conjugants. While the first gave rise to but few cultures containing conjugants and while these were produced early in its history, it is clear that it too was un-

stable; for one of the split pairs derived from the first culture gave rise to two cultures both of which contained conjugants. Following the reasoning given on pages 7 to 8, this shows that changes of mating type were occurring in both directions in this caryonide; and, therefore, it must have been unstable.

It appears unlikely that these 'stable' lines were produced by a second endomixis. All of these lines arose early in the history of the caryonide from which they were derived, at a time when endomixis does not ordinarily occur. Though careful watch was kept for it, no such early endomixis was found in any unstable caryonide.

Thus within unstable caryonides lines occasionally arise in which, as far as can be told, no changes of mating type take place. Apparently, this lack of change of mating type is a permanent characteristic of these lines. It is an interesting fact that, as far as the evidence goes, the mating type of a 'stable' line of an unstable caryonide is the same as that of the animals from which the caryonide was produced by endomixis. The relation of the information on stable lines to the determination of mating type in these unstable caryonides will be discussed later.

CARYONIDES OF UNCERTAIN CLASSIFICATION

Twenty-four caryonides were found which gave rise to at least one culture containing conjugants but whose behavior is not classifiable with certainty either as a lag effect or as unstableness of mating type. Since none of these caryonides were sufficiently studied, it is not possible to draw any definite conclusions from them. However, all of them could be insufficiently studied unstable caryonides. It is, therefore, not necessary to conclude that they represent new and as yet unrecognized types of exceptional behavior.

THE INHERITANCE OF MATING TYPE AT ENDOMIXIS IN UNSTABLE CARYONIDES

Two experiments were performed in which endomictics were obtained from unstable caryonides. In all, 113 exendo-

mictic caryonides were examined in these experiments. Not all of these caryonides were tested for mating type, but it is known that caryonides of both mating types were produced.

Thirteen caryonides were found which gave rise to at least one culture that contained conjugants and, therefore, both mating types. Eight of these appear to owe their behavior to a lag in phenotypic change such as described in a previous paper (Kimball, '39). Conjugants were found only in the first left-over cultures of these eight caryonides, and all subsequent cultures that were tested reacted solely as type I. Both members of the one split pair derived from each of two of these caryonides gave rise to cultures which reacted solely as type I. As these caryonides arose at endomixis in unstable caryonides, both type I and type II animals could have been present in the cultures that underwent endomixis. Although the mating type of the individuals from which these caryonides arose is not known directly, it could have been type II; and so the behavior of these eight caryonides could be explained as a lag effect such as described by Kimball ('39).

Only one of these caryonides which gave rise to cultures containing conjugants was clearly an unstable one. This caryonide gave rise to four successive cultures containing conjugants and therefore both mating types. Moreover, the cultures from the members of two split pairs all contained conjugants. Therefore, changes of mating type must have occurred in both directions in this caryonide and so it is clearly an unstable one. Four other caryonides that gave rise to at least one culture containing conjugants may have been unstable. However, they were too incompletely studied to allow a certain classification.

Therefore, no more than about 4% of the caryonides produced by endomixis within an unstable caryonide are themselves unstable. This is not very different from the maximum of 3% produced by endomixis within ordinary caryonides. Therefore, it is clear that unstable caryonides do not tend to give rise at endomixis to an appreciably higher per

cent of their own kind than do other caryonides. Indeed in every respect, they seem to be like other caryonides in their behavior at endomixis.

DISCUSSION

In from 1 to 3% of the caryonides produced by endomixis, changes of mating type occur repeatedly during vegetative reproduction. It is clear, however, that the type of each individual animal is not redetermined at random. How the type of an animal is determined is not clear. It may be that it is determined by the environment as found by Moewus ('35) for the sex of the gametes of the monoecious clones of the alga Protosiphon botryoides. On the other hand, some internal mechanism such as unequal nuclear or cytoplasmic division might be in operation. However, there is no evidence that allows a decision to be made in this matter.

In a few lines of these unstable caryonides, apparently, no changes of mating type ever occur. It may be that the mating type of these lines is determined by the environment, and for some reason effective changes of environment no longer occur in them. However, there is some evidence that these stable lines are determined by some internal mechanism and are different in some way from other lines. The evidence on this matter is the relationship found between the mating type of the stable lines of a caryonide and that of the animals from which the caryonide was produced by endomixis. The evidence, though meager, indicates that the mating type of the one is always the same as that of the other. If this is so, it seems necessary to assume that some internal factor is involved in the production of stable lines. As has been mentioned, cases of temporary stability have been found in which one isolation line gave rise first to a culture composed of animals of but one type and then to others composed of animals of both types. No relation was found between the mating type of temporarily stable lines of an unstable caryonide and that of the animals from which the caryonide was produced by endomixis. There thus is some evidence that

temporary and apparently permanent stability are different things.

In certain other ciliate protozoa, conjugation has been found in the absence of endomixis among the vegetative descendants of a single individual. Thas has been found by Calkins ('19) in Uroleptus mobilis, Woodruff and Spencer ('24) in Spathidium spathula, Cohen ('34) in Euplotes patella, and Woodruff ('35) in Blepharisma undulans. If mating types are involved in the conjugation of these ciliates, this would indicate that change of mating type during vegetative reproduction, which is the exception in stock S of Paramecium aurelia, may be the rule in other species or stocks.

SUMMARY

1. A few rare caryonides were found which repeatedly gave rise to cultures in which conjugation occurred.

2. The conjugation in these cultures was shown to be due to the presence in them of animals of both mating types.

3. Since these cultures were derived from single animals, changes of mating type must have occurred.

4. These changes of mating type are not caused by the repeated occurrence of endomixis.

5. The change of mating type occurs in both directions (from type I to type II and from type II to type I) and is reversible.

6. The frequency of change of mating type may be variable but in some cases can be no more than one change in every 110 animals.

7. So far as the evidence goes, cultures derived from individuals of known mating type are in most cases composed mostly of animals of the same type as the individuals from which they were derived.

8. Because of the low rate of change found in some lines and because animals tend to have descendants of the same mating type as themselves, it is concluded that the mating type of each individual animal is not redetermined at random.

9. Within these unstable caryonides, lines occasionally arise in which no changes of mating type ever occur.

10. Endomixis within unstable caryonides does not produce a greater proportion of new unstable caryonides than does endomixis within ordinary caryonides. Indeed, there seems to be no difference between the results of endomixis in these two kinds of caryonides.

11. It is not possible to decide whether the mating type of each individual animal of an unstable caryonide is determined by environmental or internal factors.

12. As far as the evidence goes, the mating type of a 'stable' line of an unstable caryonide is always the same as that of the animals from which the caryonide was produced by endomixis. If this is so, the mating type of such stable lines must be determined by internal factors.

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